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Research report

Relevance of the metabotropic glutamate receptor (mGluR5) in the regulation of NREM-REM sleep cycle and homeostasis: Evidence from mGluR5 (−/−) mice

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HIGHLIGHTS

- mGluR5 (−/−) mice showed reduced REM sleep during the light phase.
- mGluR5 (−/−) mice had maintained reduction of NREM sleep-REM sleep state transitions.
- mGluR5 (−/−) mice display enhanced motor behavior and body temperature during the dark phase.
- mGluR5 (−/−) mice exhibited reduced slow wave activity and sleep drive after sleep deprivation.
- mGluR5 is important for the stability of NREM-REM sleep cycle and sleep homeostasis.

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ABSTRACT

Sleep is a homeostatically regulated behavior and sleep loss evokes a proportional increase in sleep time and delta slow wave activity. Glutamate and pharmacological modulation of the metabotropic glutamate receptors (mGluR) signaling have been implicated in the organization of vigilance states. Here, the role of the mGluR5 on homeostatic regulation of sleep-wake cycle and electroencephalographic (EEG) activity was examined in mGluR5 (−/−) mice. We first characterized the sleep-wake EEG phenotype in mGluR5 (−/−) and wild-type (WT) littermates mice by continuous recording for 72 h of EEG, body temperature (BT) and locomotor activity (LMA). Next, we investigated the influence of sleep deprivation on the recovery sleep and EEG slow wave activity (1–4 Hz) during NREM sleep to assess whether mGluR5 deletion affects the sleep homeostasis process. Like the control animals, mGluR5 (−/−) mice exhibited a clear-cut circadian sleep-wake architecture, however they showed reduced REM sleep time during the light phase with shorter REM sleep bouts and reduced state transitions in the NREM sleep-REM sleep cycle during the first and last 24 h of the spontaneous 72 h recording period. In addition, mGluR5 (−/−) mice had decreased slow EEG delta power during NREM sleep and enhanced LMA associated with elevated BT during the dark phase. Moreover, mGluR5 (−/−) mice exhibited reduced slow wave activity and sleep drive after sleep deprivation, indicating altered sleep homeostatic processes. The findings strongly indicate that mGluR5 is involved in shaping the stability of NREM sleep-REM sleep state transitions, NREM slow wave activity and homeostatic response to sleep loss.

1. Introduction

Glutamate, the main excitatory neurotransmission in the brain, plays a major role in the control of neuronal activity [1]. Glutamate can exert its effects via the ionotropic and G protein-coupled metabotropic receptors (mGluR) including postsynaptic mGluR5 [2,3]. mGluR5 is widely expressed throughout the central nervous system and has been implicated in the modulation of neuronal function and synaptic plasticity through its interaction with *N*-methyl-D-aspartate (NMDA) receptors [4,5]. mGluR5 signaling is involved in several neurophysiological processes and its dysfunction is implicated in a multitude of psychiatric and neurological disorders, which make this receptor an exciting novel drug target for the treatment of schizophrenia, Fragile X syndrome and Alzheimer's disease [6–10].

Glutamate release shows rhythmic fluctuations, with peak levels reached during both waking and rapid eye movement (REM) sleep and lower levels reached during non-rapid eye movement (NREM) sleep [11–13]. The distribution of mGluR5 in key cortical structures involved in the control of vigilance states may suggest

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a possible role of mGluR5 in sleep mechanisms. mGluR5 is functionally linked to the Homer family of proteins which function as adaptors required for maintenance of behavioral state and consolidation of sleep and wake in both Drosophila and mouse [14,15]. It has been an open question as to what extent pharmacological modulation of the mGluR5 signaling would affect sleep-wake architecture. Recently, pharmacological manipulation of the mGluR5 signaling revealed an implication of mGluR5 in the organization of vigilance states. Activation of mGluR5 signaling elicits neuronal excitability and promotes cortical arousal [16–18], while inactivation of mGluR5 signaling enhances sleep [16,19].

The present investigations were undertaken to investigate rhythmic and homeostatic regulation of sleep in mGluR5 (−/−) mice. We first characterized vigilance states, spectral dynamics and circadian rhythms of LMA and BT in mGluR5 (−/−) mice during 72 h recording of spontaneous activity. Afterwards, we compared the cortical EEG response in the mGluR5 (−/−) and wild-type (WT) mouse littermates after sleep deprivation.

2. Materials and methods

2.1. Animals

mGluR5 (−/−) mice used in the present experiments were purchased from Jackson Laboratory (B6,129-Gprc1etm1Rod; Bar Harbour, ME, USA). Littermate wild-type (WT) mice "mGluR5 (+/+)" used to control for mixed genetic background were generated from internal breeding after seven heterozygous × heterozygous backcrosses. Mice between the ages of 8 and 12 weeks were transferred to our laboratory animal facility, where they were singly housed in a soundproof holding room with controlled environmental conditions throughout the study: 22 ± 2 °C ambient temperature, relative humidity 60%, standard 12:12 h light cycle regime (lights on at 12:00 am, illumination intensity: ∼100 lux) with standard rodent pellets and tap water were provided ad libitum. All efforts were made to minimize animal use and suffering under an AAALAC-approved animal facility that meets Federal and State requirements for care and treatment of laboratory animals. The experimental procedures were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986, and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC) or the National Institutes of Health guide for the Care and Use of Laboratory animals (NIH Publications No. 8023, revised 1978), and received approval from the animal care and use committee of Janssen Pharmaceutica Research & Development.

2.2. Surgical procedure

A total of 30 male mice (14 mGluR5 (−/−) and 16 WT mouse littermates) were surgically instrumented with the telemetry transmitter TA10ETA-F20 (Data Science International, USA) containing biopotential electrodes for the recording of EEG, EMG, BT and LMA. All surgical procedures were performed under deep isoflurane anesthesia (4% induction, and 1.5 l maintenance) with animal body temperature thermostatically controlled using the heating pad, throughout the operation and following recovery. The animals received 0.3 ml antibiotic (Albipen, ampicillinum anhydricum 100 mg/ml, Mycofarm Ireland Ltd, Dublin), and the probe was placed in the abdomen and sutured to the muscle wall, with three sutures, to prevent internal movements. The four leads coming from the telemetric probes were tunneled from the peritoneal cavity and led subcutaneously to the head in order to insert a pair of multistranded stainless-steel wires in the neck extensor muscle for recording the electromyogram (EMG) activity, and to attach the other pair ipsilaterally with stainless-steel screws for monitoring the electroencephalogram (EEG). The abdominal skin was sutured with uninterrupted stitches and animals were placed in the stereotaxic frame. An incision of 1 cm was made over the head skin in order to prepare the cortical bone surface by scalping the periosteum. The parietal EEG electrodes were placed at AP-2.3 mm, L ±1.4 mm relative to Bregma, according to stereotaxic atlas of Paxinos and Franklin [20]. The screws were affixed with dental cement to the cranium and the skin was sutured together. After surgery, the animals were subcutaneously given 0.3 ml analgesic (Carprofen, Rimadyl, 50 mg/ml, Pfizer Ltd, UK), and local analgesic (Lidocaine spray Xylocaine, 1% solution, Astra Pharmaceuticals Ltd, UK) was applied on the wounds. The mice were individually placed in their home cages and kept warm in a heating box set at 26 ± 2 °C to avoid hypothermia. The temperature was progressively decreased over days to reach room temperature. The animals had free access to food pellet and water and were allowed at least 2 weeks to recover from surgery.

2.3. Recording procedure

After 2–3 weeks recovery period and adaptation to the recording conditions, spontaneous sleep-wake cycle and circadian rhythms of BT and LMA monitoring were performed in 12 light:12 dark cycles. Recordings started by placing the animals in their home cages under the appropriate receivers. The telemetry signals were processed for analog output by an analog converter (Dataquest ART 2.3 Gold version). BT and LMA were sampled every 10-s and were averaged into 1-h bins. The EEG signals were digitized at a sampling rate of 200 Hz.

2.4. Total sleep deprivation

To examine the integrity of sleep homeostasis, mGluR5 (−/−) mice and WT mouse littermates were sleep deprived for 8 h using an automated sleep deprivation unit (Part # 8229, Pinnacle Technology, Inc.). The Pinnacle system and software allows the experimenter to control and adjust the rotation speed of the bar in order to keep an animal awake and move around the bar as it rotates.

Here, mice were sleep deprived by continuous slow rotating bar for 8-h (10 rpm), which has been proved to be compatible with classical sleep restriction paradigms [21].

The EEG/EMG were continuously recorded over 48 h, in mGluR5 (−/−) and WT mouse littermates (n = 6–8, each genotype), in which the first 24 h represents a baseline recording. During the second 24 h, animals from each genotype were sleep deprived at the light onset for 8 h and EEG/EMG were recorded during the remaining recovery period of 16 h.

2.5. Determination of vigilance states and spectral analysis

Offline, the sleep-wake phenotype and physiological variables were characterized in mGluR5 (−/−) and their WT mouse littermates over 72 h recordings of spontaneous activity. BT and LMA expressed as movements in the cage were averaged in 1-h bins. The signals were digitized at a sampling rate of 200 Hz, imported offline into Neuroscore software (Neuroscore, DSI) and digitally filtered (high-frequency band 50 Hz and low-frequency band at 0.5 Hz) while analyzing the vigilance states. The vigilance states were analyzed in consecutive 4 s epochs (Neuroscore Software, Data Sciences International) as being wakefulness, NREM sleep or REM sleep on the basis of EEG/EMG and LMA signals and according to the standard criteria. Wakefulness is characterized by a low-amplitude EEG signal with mixed frequencies associated with high and variable EMG activity. NREM sleep is dominated by high EEG amplitude, with both delta (1–4 Hz) and low EMG activity. REM

sleep is characterized by regular EEG theta oscillations (5–9 Hz) and low-amplitude EMG activity. Next, different sleep variables were calculated such as total time spent awake and asleep, number of episodes and mean duration of episodes for each vigilance state.

EEG spectral power was analyzed during both light and dark phases of the first 24 h of baseline condition. Fast Fourier transform analysis computed the EEG power in consecutive 4 s epoch within the frequency range of 0.5–50 Hz and data were collapsed in 1 Hz bins. The artifact-free power density obtained in each vigilance state was summed over the frequency (total power) and all power spectral densities in every Hz were expressed as percentage relative to total power.

2.6. Sleep recovery after sleep deprivation

The effects of sleep deprivation on recovery sleep time in both mGluR5 (−/−) and WT controls was determined as accumulated difference from baseline conditions, for consecutive hours starting at light onset. Accumulated sleep and NREM sleep delta power was compared with non-sleep deprived mice in each genotype, to assess whether mGluR5 deletion affects the recovery and homeostatic processes, which regulate the need for sleep in this mouse strain. EEG delta power density during NREM sleep was averaged in the frequency bins from 1 to 4 Hz and values were normalized and expressed as percentage of the individual mean delta power over 4 s NREM sleep epochs reached over the last 4 h of the light period when delta power is minimal during baseline in each subject.

2.7. Statistical analysis

The time course of different sleep variables under spontaneous conditions and after sleep deprivation were expressed as the mean ± S.E.M. within each treatment group and were presented as mean values for each hour over the recording sessions (72 h for the spontaneous recordings; 24 h for the basal condition prior sleep deprivation and 16 h after sleep deprivation). The mixed model ANOVA model and the Mann–Whitney tests with Bonferroni corrections for multiple comparisons were used to compare changes in sleep parameters across group. Comparisons of number of sleep-wake bouts mean duration and state transitions were made by using a non-paired two-tailed Student's *t*-test.

3. Results

3.1. Baseline conditions

3.1.1. mGluR5 (−/−) mice had reduced REM sleep during the light phase and enhanced waking associated with BT/LMA response during the dark phase

To characterize the role of mGluR5 in the regulation of vigilance states, sleep-wake cycle, BT and LMA variables were measured over the 72 h recording period in mGluR5 (−/−) and WT mouse littermates. mGluR5 (−/−) mice were rhythmic under the light-dark cycle, as all variables showed a clear-cut circadian pattern, with lowest levels occurring during the active/dark phase while highest levels during the inactive/light phase of the circadian time (Fig. 1). The distribution and amounts of waking, NREM sleep and REM sleep were similar for both genotypes during the light and dark phases of 72 h recording period. However, quantitative or qualitative changes between strains were detected in REM sleep, LMA and BT parameters (Fig. 1). During the light phase of the circadian time, mGluR5 (−/−) mice showed less REM sleep compared to age-matched WT mouse littermates. During the dark phase of the circadian time, mGluR5 (−/−) mice exhibited consistent increases in body movement associated with increases in body temperature (Fig. 1).

Analysis of vigilance-state parameters in the light period showed that mGluR5 (−/−) mice spent significantly less time in REM sleep. The amount of NREM sleep was slightly enhanced in this mutant.

To determine the stability of the sleep-wake cycle across the light dark phases, we examined the number and mean duration of individual bouts of waking, NREM sleep and REM sleep, as well as state transitions during the first and last 24 h of the entire 72 h registration period. The incidence of state transitions in the sleep wake cycle was determined by the number of transition between different vigilance states over 12 h light/12 h dark phases.

The mGluR5 (−/−) mice had longer average NREM sleep bout duration with the same number of bouts during the light segment when sleep predominates, whereas REM sleep bouts were shorter during both light and dark phases of the first and last 24 h of the entire 72 h recording period (Fig. 2A and B). During the dark phase, mGluR5 (−/−) exhibited longer waking bouts associated with enhanced arousal phenotype of this mutant (Fig. 2B).

The number of state transitions from wakefulness to NREM sleep was reduced in the light phase (Fig. 2C). There was a tendency for decreases in state transitions from NREM sleep to REM sleep and consequently from REM sleep to NREM sleep. Transitions from REM sleep to wakefulness were also reduced (Fig. 2C). Similar tendencies of decreased transitions from NREM sleep to REM sleep and from REM sleep to NREM sleep were found in the dark phase. These results indicate that differences observed during the first 24 h were still apparent in the last 24 h baseline period, which suggests that mGluR5 (−/−) mice differed from their age matched WT mouse littermates in the regulation of state transitions between NREM sleep and REM sleep.

3.1.2. mGluR5 (−/−) mice had enhanced gamma oscillatory activity during waking and reduced delta oscillatory activity during NREM sleep

The effect of the mGluR5 mutation on EEG dynamics was examined in different vigilance states during the 12 h light:12 h dark cycle. Higher frequency oscillations particularly in the gamma frequency range were significantly higher in waking during both the light and dark phase of the circadian time (Fig. 3).

Compared with WT animals, mGluR5 (−/−) mice had relatively lower NREM sleep delta activity notably during the dark phase of the circadian time. The distribution of mean EEG power spectral density during wakefulness showed an increased power of over 20 Hz, whereas a shift in peak power density from 7 Hz to 5–6 Hz was found in REM sleep during the light phase.

3.2. Sleep deprivation conditions

3.2.1. mGluR5 (−/−) mice exhibited altered sleep homeostatic response after sleep deprivation

mGluR5 (−/−) mice that have not been deprived of sleep had reduced REM sleep state and more NREM sleep when compared to WT mouse littermates in the same condition. When released from sleep deprivation over the 8 h period during the light phase, both genotypes spent less time awake during the dark period of the circadian time. However, there were consistent differences in NREM sleep and REM sleep during the dark phase between the genotypes (Fig. 3A). During the recovery sleep period, a sharp surge of NREM sleep was found in both genotypes, which protruded into the dark period. WT mouse littermates progressively recovered REM sleep particularly during the dark period. In contrast, mGluR5 (−/−) mice had no recovery REM sleep either during the light or the dark period, indicating that a homeostatic drive for REM sleep was lacking in these mutant mice.

Slow wave activity: To determine whether other indices of a homeostatic response were affected in mGluR5 (−/−) mice, we

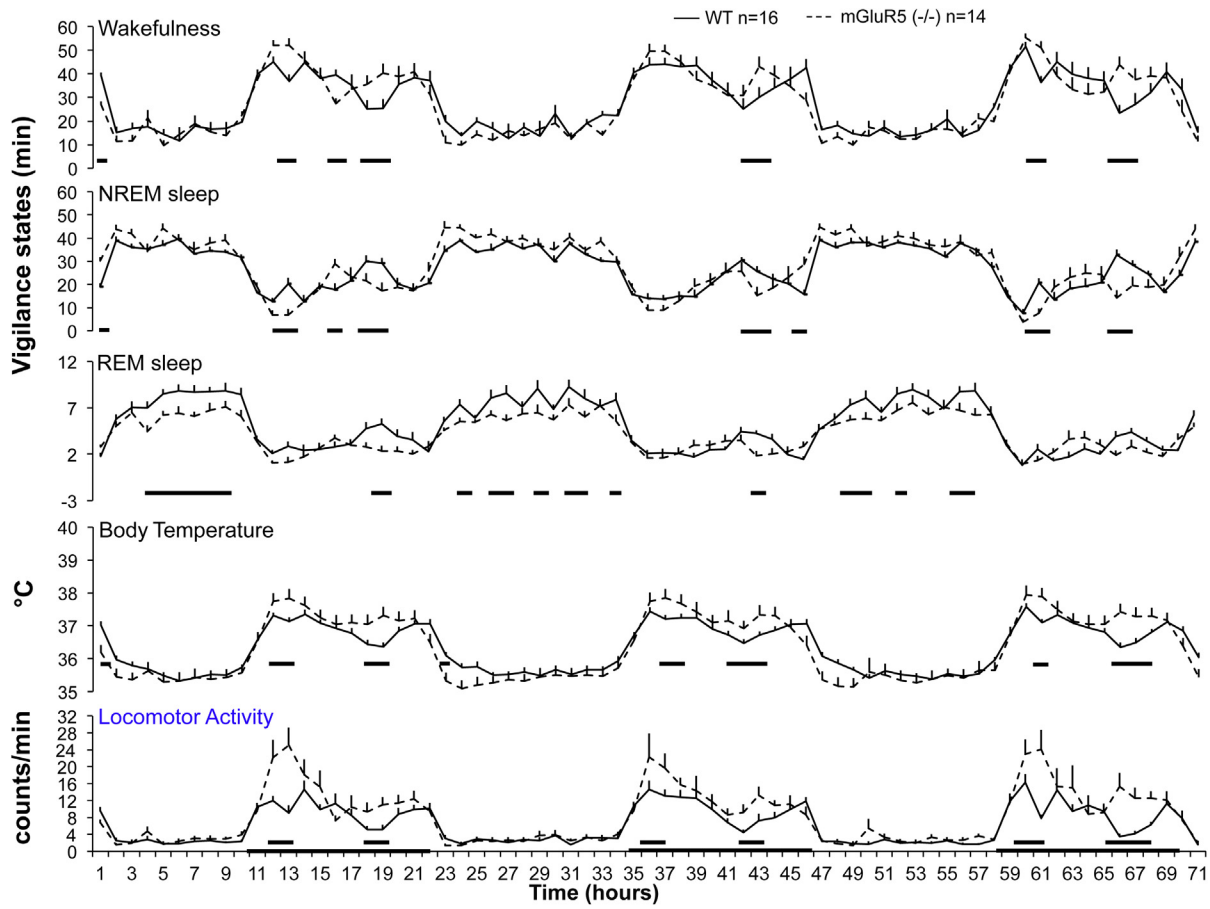

Fig. 1. Seventy-two hours spontaneous sleep-wake profiles, body temperature (BT) and locomotor activity (LMA) under baseline conditions in mGluR5 (−/−) (n = 16) and WT mouse littermates (n = 14). Comparison of the percent time spent in waking, NREM, REM sleep, BT and LMA averaged over 1 h blocks in mGluR5 (−/−) (dotted lines) and WT mouse littermates (bold lines). Significant differences were found among the genotypes during the light phase in REM sleep, and during the dark phase for waking, NREM sleep, body temperature and locomotor activity. Black horizontal bars in the abscissa axis indicate the 12 h dark phase of the circadian time. Black bars underneath the curves indicate $p < 0.05$; mGluR5 (−/−) versus WT mouse littermates.

analyzed the slow wave activity (0.5–4 Hz), which is considered to be a more sensitive index of sleep drive. A progressive increase during the second part of the light period and during the dark period was found in all genotypes. An increased delta power that was longer during the recovery period was found in WT mouse littermates (Fig. 4B). However, a weak increase in slow wave activity was revealed in mGluR5 (−/−) mice indicating impairments in sleep drive and homeostatic regulation of sleep in this mutant.

4. Discussion

The present study used mGluR5 (−/−) mice to determine the consequences of permanent deletion of the mGluR5, on the organization of vigilance states and homeostatic response to sleep loss. The findings indicate that mGluR5 is required for the regulation of the daily amounts and stability of NREM-REM sleep states as well as sleep homeostasis in this mutant.

4.1. Characterization of vigilance states in mGluR5 (−/−) mice

Under baseline conditions, mGluR5 (−/−) mice displayed a rich sleep-wake phenotype. On the one hand, the mGluR5 (−/−) animals had a normal circadian sleep pattern and related physiological parameters expressed a clear diurnal rhythm after 2 weeks of adaptation to the recording conditions. Large amount of waking occurred during the dark active phase, whereas sleep dominated the light inactive phase of the circadian time. The mGluR5 mutation did not affect the phase position of sleep-wake states and the circadian rhythms appear to be unaltered in mGluR5 (−/−) mice, suggesting that mGluR5 is not directly involved in the regulation of circadian processes.

On the other hand, an enhancement of NREM sleep and impairment in the genesis of REM sleep were revealed in the mGluR5 (−/−) mice. Recent pharmacological studies demonstrated that pharmacological modulation of the mGluR5 signaling affected the organization of vigilance states. Allosteric positive modulation of the mGluR5 elicited arousal and decreased sleep in rats [16–18], whereas allosteric negative modulation of mGluR5 significantly reduced REM sleep and enhanced sleep in rats [16–19,22].

Wakefulness and sleep are regulated by several parallel mechanisms. Wakefulness is promoted by the ascending arousal systems such as acetylcholine, serotonin, norepinephrine, dopamine, histamine, glutamate and orexin systems [23]. Sleep is controlled by the descending inhibitory sleep pathways, of which the ventrolateral preoptic area (VLPO) GABAergic, adenosinergic and glycinergic systems are primarily involved in the regulation of NREM sleep, and the brainstem laterodorsal and pedonculopontine tegmentum nuclei, are involved in REM sleep generation [23].

As a postsynaptic receptor, mGluR5 interacts with NMDA receptors and components of the ascending and/or descending systems to modulate neuronal activities and vigilance states. For instance, blockade of the mGluR5 elicited consistent inhibition of norepinephrine tone in the frontal cortex, which inhibited glutamate release and reduced NMDA receptor function in a number of brain

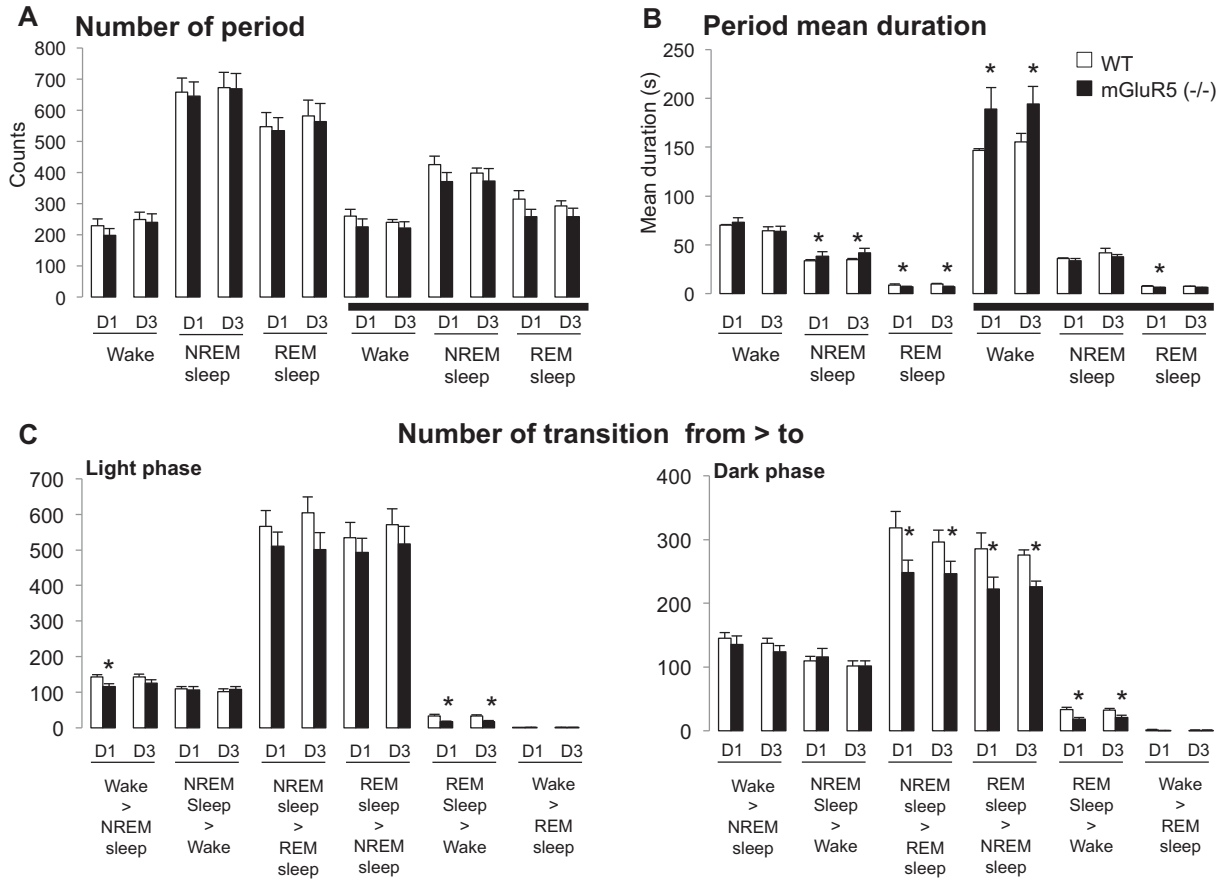

Fig. 2. (A) Number of periods, (B) mean duration and (C) state transitions during the first day (D1) and last day (D3) of the spontaneous 72 h recording period in mGluR5 (−/−) (black bars, $n = 16$) and WT mouse littermates (white bars, $n = 14$). Open and dark horizontal bars in the abscissa axis indicate the 12 h light and 12 h dark phases of the circadian time, respectively. MGluR5 (−/−) mice exhibited shorter REM sleep bouts and reduced behavioral state transitions form NREM sleep to REM sleep. *$p < 0.05$; mGluR5 (−/−) versus WT mouse littermates.

areas [24,25]. In addition, direct blockade of the NMDA receptor with MK-801 or ketamine elicited massive slow wave sleep and homeostatic recovery evoked by high metabolic activity [26,27]. Moreover, the allosteric antagonist of mGluR5 MTEP ([(2-methyl-1, 3-thiazol-4-yl)ethynyl]pyridine) has been shown to evoke 5-HT release in the hippocampus and frontal cortex [28,29], which may contribute to sleep facilitating effects following the activation of 5HT2A/2C receptors. Thus, the enhanced sleep and the deficiency in REM sleep observed in the mGluR5 (−/−) mice may derive from a chronic imbalance in the interaction of mGluR5 with components of the brain's ascending and descending systems, of which activities are known to trigger or to dampen the occurrence of NREM and REM sleep states.

Analysis of sleep parameters during the first and last 24 h of the entire 72 h recording period revealed an important role of mGluR5 signaling in the control of switches in the NREM sleep-REM sleep cycle, leading to reduced REM sleep time. MGluR5 mutant mice exhibited a lower incidence of state transitions from NREM sleep to REM sleep and from REM sleep to NREM sleep. The physiological significance of this reduced shift in NREM-REM sleep states is not yet understood. According to the cognitive concept of sleep, NREM sleep and REM sleep are both important for processing memory traces and serve distinct functions in learning and memory [30,31]. Memory traces may be destabilized during transition from NREM sleep to waking episodes, resulting in the clearing of nonessential information from the brain, whereas transition from NREM sleep to REM sleep may be important for memories to be stored again and integrated with preexisting memory traces during ensuing REM sleep episodes [30]. Consistent with cyclical notions of consolidation and reconsolidation of memory traces, replies from different neuronal ensembles during NREM sleep and REM sleep may respectively represent early and late phases of consolidation of labile memory into more stable and relatively permanent long term forms [32]. Therefore, less sequential transitions in the NREM-REM sleep cycle might correlate with impaired cognition processes described in the mGluR5 (−/−) mice [33].

In line with recent pharmacological studies, blockade of mGluR5 enhanced deep sleep and reduced the number and mean duration of REM sleep bouts in rats [16–19]. Pontine and basal forebrain cholinergic projections to the hypothalamus and cortical structures play an important role in the induction of REM sleep state [23,34,35]. We hypothesize that mGluR5-induced modulation of cholinergic neurotransmission could be relevant in the induction and maintenance of the switch from NREM sleep to REM sleep in this mutant. Extracellular recordings at pontine and basal forebrain structures is warranted to test the hypothesis, of which mGluR5-induced changes in cholinergic activity could trigger a switch from NREM sleep to REM sleep and possibly maintain the REM sleep state.

The present findings indicate that mGluR5 deficiency is closely related to the sleep-wake phenotype seen in this mutant and underscore the importance of mGluR5 in the genesis and/or maintenance of the NREM sleep-REM sleep cycle.

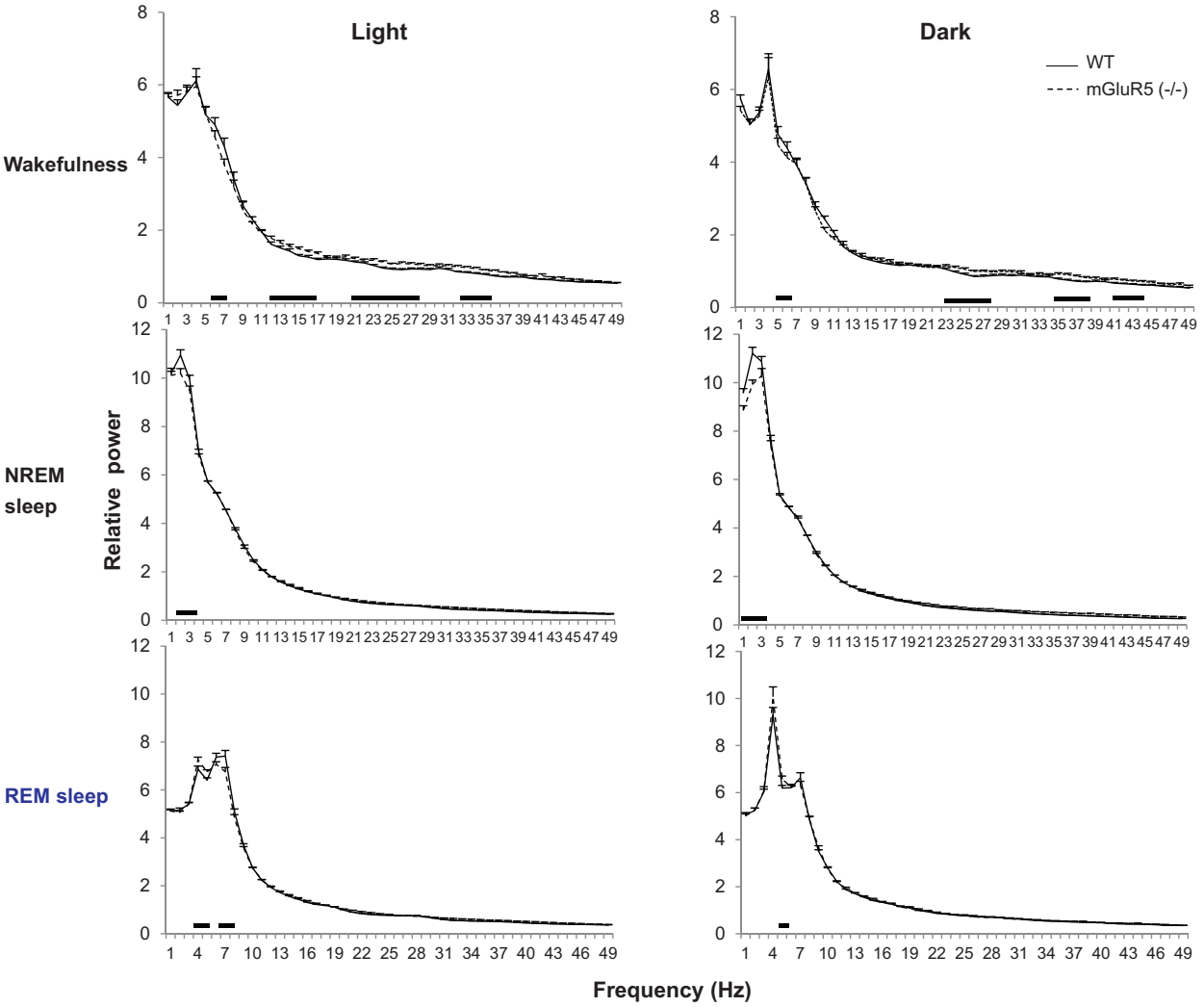

Fig. 3. EEG power spectral density during wakefulness, NREM sleep and REM sleep in both light and dark phases of the circadian time. Note the decrease in slow wave activity during NREM sleep as well as the prominent EEG gamma activity during wakefulness, in the light and dark phases of the circadian time. Black bar underneath the curves indicate $p < 0.05$; mGluR5 (−/−) versus WT mouse littermates.

4.1.1. mGluR5 (−/−) mice exhibit enhanced EEG gamma activity during waking and impaired slow wave activity during NREM sleep

Under baseline conditions, mGluR5 (−/−) mice showed prominent EEG gamma waves during waking state, in the light and dark phases of the circadian time.

The association between behavioral states and EEG oscillations has been investigated in earlier studies. Quantitative results in animal and human studies have correlated the amplitude and coherence in the EEG gamma oscillations, with attentive and active waking behavior [36,37]. Accordingly, EEG gamma activity was used as a sensitive indicator of behavioral and cortical arousal of the animal across sleep-wake states, being high in amplitude during attentive and particular active waking behaviors, and REM sleep [36]. In addition, the increased gamma oscillatory profile in mGluR5 (−/−) mice is in line with our recent pharmacological study in rats, in which allosteric mGluR5 blockade consistently enhanced oscillations and elicited prominent functional coherent network activity in the gamma frequency range [16].

The present study demonstrated significantly greater EEG gamma activity during wakefulness reflecting an arousal phenotype in the mGluR5 (−/−) mouse strain.

mGluR5 (−/−) mice exhibited altered EEG slow wave activity during NREM sleep. Synchronization of the EEG during NREM sleep depends on thalamic as well as intrinsic cortical oscillations, and reduced neuronal excitability and synaptic transmission facilitates slow waves leading to neuronal down-states [38]. The enhanced NREM sleep time in mGluR5 (−/−) mice, which resulted from longer average bout duration with the same number of bouts may suggest that there are relatively fewer shorter bouts of NREM sleep initiated, because sleep drive was lowered due to the increased NREM sleep duration. This deficit in slow wave activity is consistent with the reduced delta power following sleep deprivation in this mutant. Thus, mGluR5 may be necessary to maintain the synchronization of cortical neuronal networks that underlies EEG rhythm in the delta range. Here, mGluR5 (−/−) mice were quantitatively deficient in slow wave activity, which may have detrimental consequence on alertness; however their waking state during both light and dark phases was associated with enhanced gamma network oscillations suggesting compensatory signs of enhanced vigilance during waking state.

During REM sleep, there was a left-ward shift in the EEG power density curve of theta rhythm from 7 Hz in the WT mice to 5–6 Hz in the mGluR5 (−/−) mice.

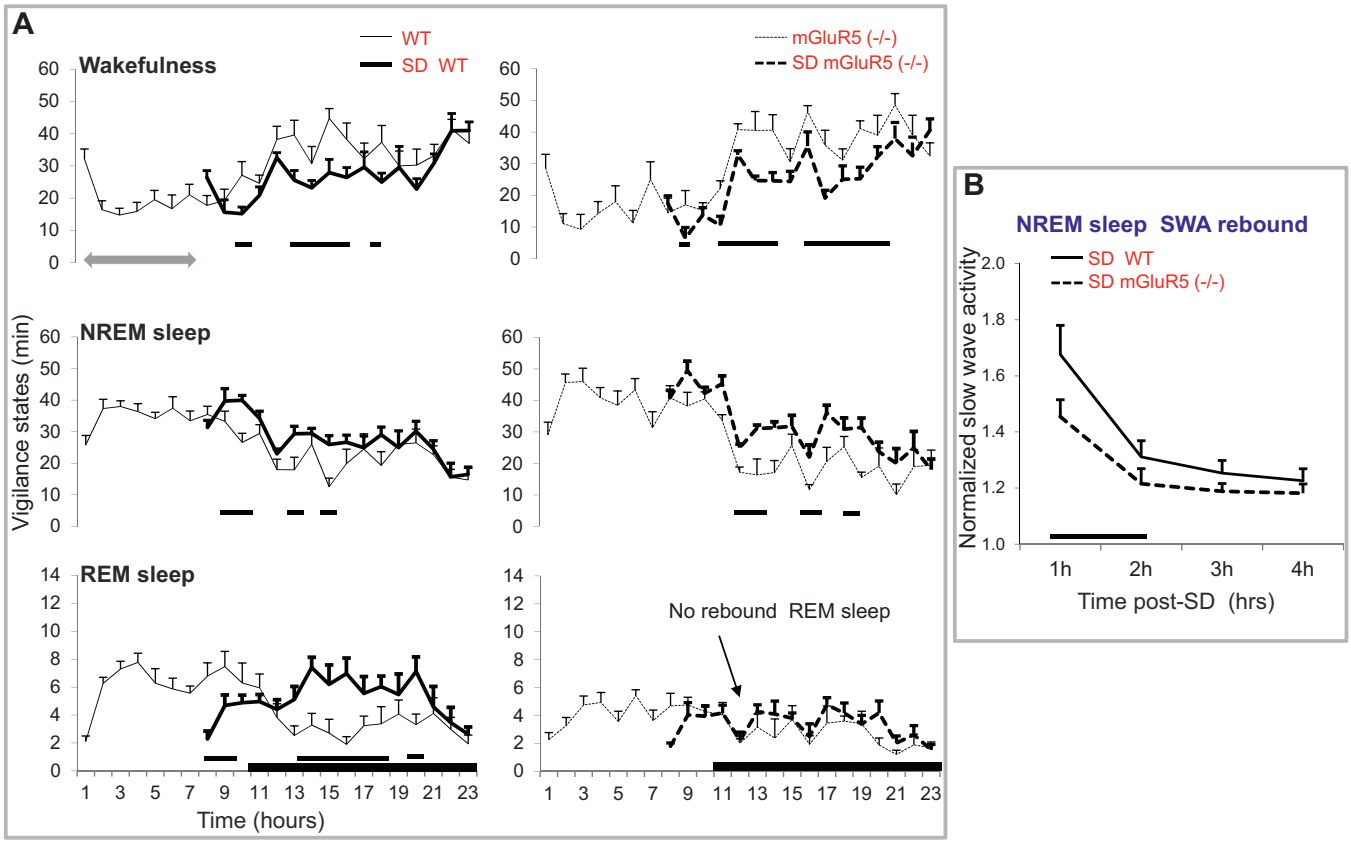

Fig. 4. Effects of 8 h sleep deprivation during the first part of the light period on (A) duration of NREM sleep and REM sleep recovery and, (B) NREM sleep slow wave activity (SWA) rebound. During the baseline day prior sleep deprivation, mGluR5 (−/−) mice (thin continuous curve) had sleep-wake profile as observed in the first set of phenotypic experiment, i.e. low REM sleep and high NREM sleep levels as compared to controls (thin dashed curve). A consistent recovery of both NREM sleep and REM sleep was observed in WT mouse littermates during the next 16 h that followed sleep deprivation (Part A). However, mGluR5 (−/−) mice failed to show such recovery sleep, particularly for REM sleep indicating that a homeostatic drive was altered in this mutant. During the recovery sleep period, all genotypes had increased NREM sleep SWA rebound, however the increase was short lived in mGluR5 (−/−) and longer in WT mouse littermates (Part B). SD indicates sleep deprivation and the gray arrow on the abscissa axis in part A indicates the interval of sleep deprivation. Black bars underneath the curves indicate $p < 0.05$; mGluR5 (−/−) versus WT mouse littermates.

The mean firing rate during waking and sleep showed significant state-related changes in the population of hippocampal single neurons activity [39]. A significant reduction in burst neuronal firing rate is observed during REM sleep. It is possible that discharge variability may influence the highly rhythmic bursting that accompanies theta rhythm during REM sleep in mGluR5 (−/−) mice, which may result in redistribution and shift in bursting activity at a slower rate theta frequency. These findings indicate that mGluR5 signaling is involved in the modulation of delta and theta oscillatory activities.

4.2. *mGluR5 (−/−) mice exhibit enhanced LMA and BT during the dark period*

The mGluR5 (−/−) mice housed under a normal light–dark cycle, exhibited similar peaks in temperature and motor behavior relative to their WT mouse littermates, suggesting that disruption of the mGluR5 gene had no effect on the phase position of the temperature and motor rhythms. However, mGluR5 (−/−) mice exhibited hyper locomotor activity associated with increased BT during the active dark phase of the circadian time.

Although mGluR5 is present in key element of the molecular clock in the SCN, their absence did not lead to a gradual loss of rhythmicity during the 12 h light:12 h dark cycle. mGluR5 (−/−) mice had normal circadian distribution pattern of sleeping and waking states, body temperature and locomotor behavior, despite alterations in the daily amount of sleep. Mixed data have been reported regarding the motor activity in mGluR5 (−/−) animals. Initial studies found no difference in locomotor activity [33,40], whereas a recent work demonstrated a hyperactive phenotype in mGluR5 (−/−) mice, which could be potentiated by the non-competitive antagonist MK-801 [41]. There is evidence indicating that the serotonin 5-HT2A receptor function may be regulated by mGluR5 [42,43]. The mGluR5 (−/−) mice have shown an increased behavioral response to the hallucinogenic 5-HT2A receptor agonist 2,5-dimethoxy-4-methylamphetamine (DOM) [44].

In rats, the specific mGluR5 antagonist MPEP had little effects on its own on ambulatory activity in rats; however, a consistent potentiation of PCP-induced locomotor activity was found when MPEP was combined with PCP [45]. Pharmacological blockade of mGluR5 using MTEP increased 5-HT release in the hippocampus and frontal cortex [28,29]. Given the functional interaction between mGluR5 and 5-HT2A receptors, the loss of mGluR5 signaling may enhance the sensitivity to diurnal changes in 5-HT neurotransmission and activated 5-HT2A receptors, which may result in the greater locomotor levels than expected in this mouse strain. Thus, the increased locomotor activity phenotype in mGluR5 (−/−) mice may not be solely due to an increase waking time.

4.3. *mGluR5 (−/−) mice had impaired homeostasis processes*

The duration of the recovery NREM sleep after prolonged wakefulness was increased in both genotypes; however, a weaker slow wave activity rebound was found in mGluR5 (−/−) mice, indicating that mGluR5 are critical components in the regulation of sleep pressure. The greatest increase in the slow wave activity after sleep

deprivation lasted up to 3 h of the recovery sleep period in WT mice, whereas it was selectively attenuated and shorter in mGluR5 (−/−) mice.

Slow wave activity is a powerful index of homeostatic sleep pressure that accumulates in duration and/or intensity proportional to prior wakefulness [46,47]. In addition, an increased level of extracellular adenosine in the basal forebrain during prolonged waking is a well-known marker of the homeostatic sleep drive [47,48]. A recent clinical report showed an increased expression of mGluR5 in several cortical regions after prolonged waking [49], suggesting that mGluR5 exerted a homeostatic load and increased the drive to sleep after prolonged waking. The mGluR5 are co-localized and interact directly with Homer1 proteins described as a molecular correlate of sleep homeostasis [15], which uncouples mGluR5 from effector targets to modify the homeostasis of intracellular calcium and to protect and/or to recover neuronal activity after extended period of wakefulness. Although the activity of Homer proteins and adenosinergic receptors was not measured in the present study, it may be speculated that decreased functional interaction between these systems with mGluR5, may contribute to impaired homeostatic drive in mGluR5 (−/−) mice after sleep deprivation.

Overall, the chronic lack of mGluR5 signaling altered the organization of sleep-wake states and homeostatic sleep drive, suggesting that adaptive mechanisms were unlikely to have been compensated for in this mouse strain.

4.4. Significance

Sleep is an integral component of cognition mechanisms and the increases in slow wave activity during NREM sleep is correlated with increases in synaptic plasticity events, facilitating important functional learning and memory processes [50,51]. The use of permanently mGluR5-deficient mice demonstrated a major role of this receptor in shaping the stability of NREM sleep-REM sleep state transitions, NREM slow wave activity and homeostatic response to sleep loss. Therefore, disturbance in cyclic transitions of sleep-wake states and homeostatic drive might contribute to the convergent behavioral and cognitive alterations, described in mice lacking global mGluR5 [33]. However, deletion of mGluR5 expression only in principal cortical glutamatergic neurons did not impair sensorimotor gating and several forms of learning and social interactions [52], which is encouraging and holds promise to the mGluR5 and cognition field of research. The present results outline the potential effects of mGluR5 blockade on the mechanism of sleep and warrant assessment of vigilance states when considering clinical trials with new mGluR5 drug class therapy.

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